PHYLOGENY, HISTORICAL BIOGEOGRAPHY, AND MACROEVOLUTION OF HOST USE AMONG *DIASTROPHUS* GALL WASPS (HYMENOPTERA: CYNIPIDAE)

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Abstract.—Phylogenetic relationships among 14 species of 3 Holarctic gall wasp genera, *Diastrophus* Hartig, *Gonaspis* Ashmead, and *Xestophanes* Förster (Hymenoptera: Cynipidae: Cynipinae: Aylacini), are inferred from 43 adult morphological characters. The incorporation of four characters associated with gall morphology is explored, although only a single parsimony-informative gall character is obtained. All analyses retrieve *Diastrophus* as paraphyletic with respect to the monotypic genus *Gonaspis*. These two genera share several morphological synapomorphies as well as related host plant associations. The genus *Gonaspis* is thus a **new junior synonym** of *Diastrophus*, and *Gonaspis potentillae* is herein transferred to *Diastrophus* as *D. potentillae* Bassett. In addition, *D. kincaidii* var. *austrior* Kinsey is formally elevated to specific status, as *Diastrophus austrior* Kinsey (**new status**). Association of *Diastrophus* species with *Rubus*, as well as with woody host plants, could have originated once, but the possibility of more than one origination cannot be excluded. In either case, at least two reversal events have contributed to the patterns of association of *Diastrophus* with *Potentilla*, and with herbaceous host plants. Reconstruction of the historical biogeography of the group using a dispersal and vicariance analysis model suggests that the ancestor of *Diastrophus* was either East Nearctic (EN) or Holarctic. The two West Palearctic (WP) species resulted from either two independent dispersals from EN followed by subsequent vicariance, or from a basal vicariance between WP and EN and a subsequent dispersal from EN to WP followed by a subsequent vicariance between the two regions. The West Nearctic species resulted from dispersals from EN and are relatively recent.

Key Words: biogeography, *Diastrophus, Gonaspis,* host shifting, host plant growth form, morphology, phylogeny

Extant gall-forming members of the family Cynipidae (Hymenoptera) comprise five of the six tribes in the subfamily Cynipinae, i.e., Aylacini, Cynipini, Eschatocerini, Pediaspidini, and Diplolepidini (Ronquist 1999). All the cynipid tribes except Aylacini are apparently monophyletic and appear to have relatively conserved host plant associations with host plants usually belonging to a single genus or a few closely related genera (Liljeblad and Ronquist 1998). In contrast, the tribe Aylacini, com-

prising 21 genera, is apparently an assemblage of paraphyletic and polyphyletic clades (Ronquist 1994, Liljeblad and Ronquist 1998, Ronquist 1999), and its members use a wide range of host plants belonging to Apiaceae, Asteraceae, Brassicaceae, Lamiaceae, Papaveraceae, Rosaceae, and Valerianaceae (Liljeblad and Ronquist 1998, Ronquist and Liljeblad 2001).

According to Liljeblad and Ronquist (1998), all cynipid wasps inducing galls on woody rosid plants form a monophyletic clade. These rosid gallers include the tribes of Diplolepidini inducing galls on *Rosa* (Rosaceae), Eschatocerini on *Acacia* and *Prosopis* (Fabaceae), Pediaspidini on *Acer* (Aceraceae), and the species-rich Cynipini primarily on *Quercus* (Liljeblad and Ronquist 1998, Ronquist 1999). Ronquist and Liljeblad (2001) explored various aspects of the host plant association of cynipid wasps on basis of the aforementioned phylogeny and concluded that cynipid gall wasps are 'extremely conservative' in their association with plant growth form. They further suggested that the gall wasp ancestor is likely to have been an herb galler, and colonization of woody hosts has occurred only three times, assuming that all the eight *Rubus*-galling species of *Diastrophus* Hartig form a monophyletic clade.

The genus *Diastrophus* is rather unusual among cynipid gall wasps in two aspects of their host plant associations. Firstly, *Diastrophus*, as well as *Gonaspis* Ashmead and *Xestophanes* Förster, is associated with rosaceous hosts of the genera *Rubus*, *Potentilla*, and *Fragaria* (with one species, *Diastrophus smilacis* Ashmead inducing galls on the monocotyledonous *Smilax* [Smilacaceae]), in contrast to the other Aylacini genera which induce galls on relatively advanced herbaceous host plants (Ronquist 1994). Secondly, host plants of the genus include both woody (*Rubus* and *Smilax*) and herbaceous (*Potentilla* and *Fragaria*) plants, a deviation from the generally very conservative pattern of association with plant growth form in Cynipidae. Therefore,
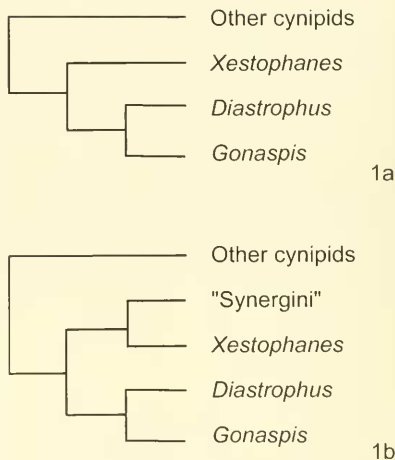
Fig. 1. Phylogenetic relationship among *Diastrophus*, *Gonaspis*, and *Xestophanes*, (a) as suggested by Nieves-Aldrey (1994), (b) as suggested by Liljeblad and Ronquist (1998).

the evolution of host plant associations in *Diastrophus* could shed light on the evolution of host use in Cynipidae in general.

Nieves-Aldrey (1994) suggested that *Diastrophus*, *Gonaspis*, and *Xestophanes* form a monophyletic clade (Fig. 1a) on the basis of their rosaceous host associations, and the presence of lobed claws and glabrous sculpture on the vertex and mesothorax (Figs. 2a–c). On the basis of phylogenetic analysis using morphological characters, Liljeblad and Ronquist (1998) partially supported this view, but suggested that *Xestophanes* and the inquilinous tribe Synergini form the sister clade of (*Diastrophus* + *Gonaspis*) (Fig. 1b). Recent molecular work (Liu et al. unpublished data) supports the view of Nieves-Aldrey (1994).

Diastrophus is morphologically distinct among Aylacini genera by the combination of strongly lobed claws, subcosta and radius reaching to anterior margin of wing; and completely free third and fourth abdominal terga in both sexes (Fig. 2d, cf. *Xestophanes potentillae* De Villers, Fig. 2e) (Nieves-

Aldrey 1994), and thus obviously monophyletic. It is not clear whether *Gonaspis*, which comprises only one known species, should be included within *Diastrophus* or is indeed a sister clade of *Diastrophus*. Ronquist (1994) and Liljeblad and Ronquist (1998) listed five synapomorphies for *Diastrophus+Gonaspis*. However, these studies, for their respective purposes, each included only one *Diastrophus* species and were not able to clarify the relationship among *Gonaspis* and *Diastrophus* species. In fact, only a single morphological feature separates the monotypic *Gonaspis* from *Diastrophus*: the scutellum of *Gonaspis* conspicuously projected posteriorly (Fig. 2f). This is an autapomorphic character, for which an intermediate form exists in *D. cuscutaeformis* Osten Sacken (not illustrated).

Biologically, the members of *Diastrophus*, as well as those of *Gonaspis* and *Xestophanes*, are univoltine and lack the alternation of generations (heterogony) found in the more derived oak-galling cynipids. Like galls induced by other wasps of the tribe Aylacini, their galls are mostly simple swellings on stems, leaves, and roots, and lack complicated secondary external structures. Cynipid wasps lumped in the tribe Aylacini invariably make galls not detachable from the host plant. This is also true for *Gonaspis*, *Xestophanes*, and most species of *Diastrophus*, regardless of host form, i.e., woody versus herbaceous. Only one species of *Diastrophus*, *D. cuscutaeformis*, makes detachable galls, as do some Cynipini gallers of woody plants. In addition, some members of *Diastrophus* associated with woody *Rubus* bushes are attacked by inquilines of the cynipid genus *Synophromorpha* Ashmead. Inquilines of cynipid gall wasps do not induce galls of their own, but instead live in and feed on galls induced by the other cynipid wasps. Only galls on woody host plants are known to be attacked by inquilines.

While *Gonaspis* is a monotypic genus endemic to the Nearctic and *Xestophanes* includes only two species from the West Palearctic, the genus *Diastrophus* (Hymenoptera: Cynipidae: Aylacini) comprises sixteen described species distributed throughout the Holarctic. Known species of *Diastrophus* include two from the West Palearctic (*D. mayri* Reinhard 1877 and *D. rubi* [Bouché 1834]) and one undescribed species from Japan in the East Palearctic identified on the basis of the presence of *Diastrophus* galls and rearing of the host-specific *Synophromorpha* Ashmead inquilines from these galls (Abe 1998, Abe personal communication); one described nominate subspecies (*D. k. kincaidii* Gillette 1893) and its heteronomic subspecies (*D. kincaidii austrior* Kinsey 1922) from the West Nearctic; and twelve East Nearctic species (*D. bassetti* Beutenmüller 1892, *D. cuscutaeformis* Osten Sacken 1863, *D. fragariae* Beutenmüller 1915, *D. fusiformans* Ashmead 1890, *D. minimus* Bassett 1900, *D. nebulosus* (Osten Sacken 1861), *D. niger* Bassett 1900, *D. piceus* Provancher 1886, *D. radicum* Bassett 1870, *D. smilacis* Ashmead 1896, *D. tumefactus* Kinsey 1920, and *D. turgidus* Bassett 1870). The two European species of *Diastrophus* Hartig 1840 (Hymenoptera: Cynipidae) were recently reviewed (Nieves-Aldrey 1994). However, there has been no revisionary study of the Nearctic species of this genus since Beuttenmueller (1909), although Burks (1979) compiled a list of all Nearctic species of the genus. Moreover, phylogenetic relationships among the *Diastrophus* species are not known and a phylogeny-based systematic revision has never been attempted.

Therefore, our goals of the present study are to reconstruct the phylogenetic relationships among *Diastrophus* species and *Gonaspis*, based on adult morphology, and use the resulting cladogram to examine (1) the macroevolution of associations between wasps and their host plants and test the hypothetical monophyletic shifting from herbaceous host plants to the woody *Rubus* host plants, (2) the historical biogeography of the genus, and (3) the phylogenetic position of *Gonaspis potentillae* in relation to
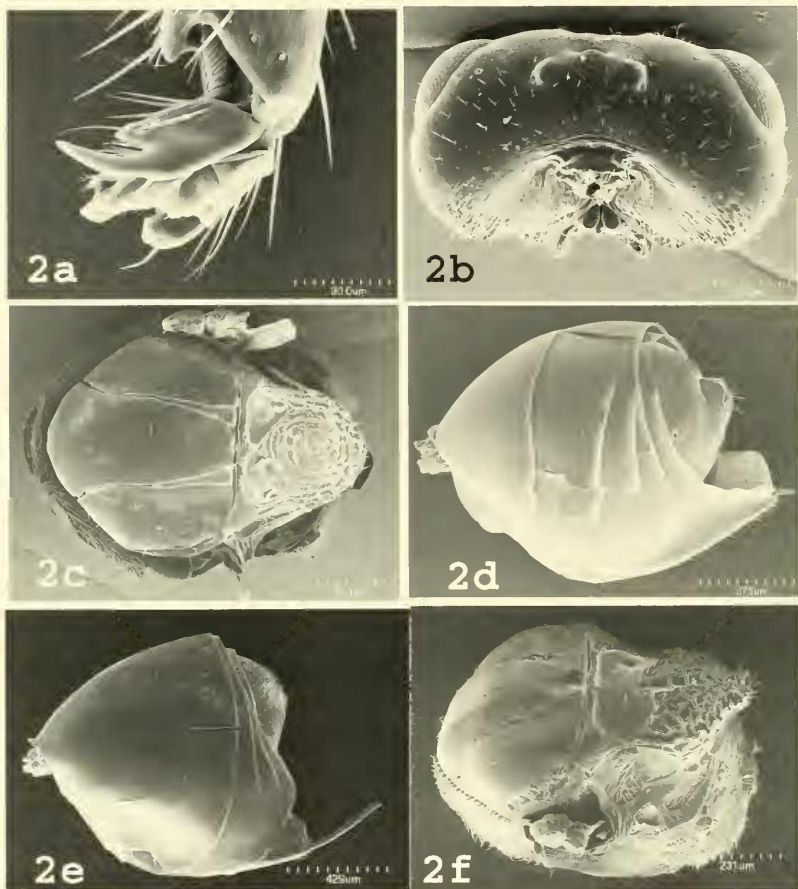
Fig. 2.   *Diastrophus* and *Xestophanes* species. (a) hind claw of *D. nebulosus*; (b) head of *D. nebulosus*; (c) mesonotum of *D. nebulosus*; (d) metasoma of *D. nebulosus*; (e) metasoma of *X. potentillae*; (f) mesosoma of *D. potentillae*.

Diastrophus species, testing the phyloge- netic independence of the monotypic genus *Gonaspis*, as discussed by Liljeblad and Ronquist (1998).

MATERIALS AND METHODS

Type specimens of 14 of the Nearctic species and the subspecies were examined,

along with additional specimens compared to types for Nearctic and Palearctic species. The undescribed Japanese species is not in- cluded in the analysis because no speci- mens of this species were reared. *Diastro- phus piceus* is also excluded from the study because the only reported material of this species is its type, which we were unable

Table 1. Taxa included in the study, their distribution and host plants. EN = East Nearctic, WN = West Nearctic, NA = Nearctic, and WP = West Palearctic. The undescribed species of *Diastrophus* from Japan, reported on basis of presence of galls, is not included in the analysis. The eastern Canadian species, *D. piceus* Provancher 1886, is not included in the study because we were unable to locate the type. References listed are in addition to original references to each species, of which all have been consulted.

Taxa	Distribution	Host Plant	References
Diastrophus austrior	WN	*Rubus*	Burks (1979)
D. bassetti	EN	*Rubus*	Burks (1979)
D. cuscutaeformis	EN	*Rubus*	Burks (1979)
D. frageriae	EN	*Fragaria virginiana*	Burks (1979)
D. fusiformans	NA	*Potentilla*	Burks (1979)
D. kincaidii	WN	*Rubus parsiflorus*	Burks (1979)
D. mayri	WP	*Potentilla argentea*	Nieves-Aldrey (1994)
D. minimus	EN	*Potentilla canadensis*	Burks (1979)
D. nebulosus	EN	*Rubus*	Burks (1979)
D. niger	EN	*Potentilla canadensis*	Burks (1979)
D. radicum	NA (mainly EN)	*Rubus occidentalis*	Burks (1979)
D. rubi	WP	*Rubus*	Nieves-Aldrey (1994)
D. smilacis	EN	*Smilax*	Burks (1979)
D. tumefactus	EN	*Potentilla*	Burks (1979)
D. turgidus	EN	*Rubus strigosus*	Burks (1979)
Gonaspis potentillae	EN	*Potentilla*	Weld (1950), Liu (field data)
Xestophanes potentillae	WP	*Potentilla*	Nieves-Aldrey (1994)

to examine. We also noted that Beuten-müller (1909) was unable to distinguish it from other *Diastrophus* species based on Provancher's original description and suggested that this might not be a valid species. The two subspecies of *D. kincaidii* were included as separate operational taxonomic unit. Loaned material included specimens from six institutions and three private collections. A total of 395 specimens were examined. Collections utilized for this study and the persons through whom the loans were kindly arranged are as follows:

AMNH   American Museum of Natural History, New York, NY, USA (J. Carpenter).
CASC   California Academy of Sciences, San Francisco, CA, USA (R. Zuparko).
CNCI   Canadian National Collection of Insects, Ottawa, ON, Canada (J. Huber).
EMEC   University of California, Berkeley, CA, USA (C. Barr).
FMNH   Field Museum of Natural Histo-

ry, Chicago, IL, USA (P. Goldstein).
MCZC   Museum of Comparative Zoology, Cambridge, MA, USA (P. Perkins).
UCDC   University of California, Davis, CA, USA (S. Heydon).
USNM   National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (D. Smith).

Other specimen contributors are:

ZL   Zhiwei Liu
OP   Olivier Plantard
FR   Fredrik Ronquist.

Morphological characters for all species were examined and identified using a Leitz Wetzlar stereomicroscope. SEM images were obtained for *Gonaspis potentillae,* *Xestophanes potentillae,* and *Diastrophus nebulosus,* using Hitachi S4700 Field Emission Scanning Electron Microscope (FE-SEM) at the AMNH. Additional SEM images were also taken for *D. mayri, D. niger,*

and *D. turgidus* using an Amray 1810 at the FMNH to assist character coding. Mounted specimens were first transferred to and kept in 70% ethanol for weeks, dissected in 70% ethanol, cleaned with kitchen ammonia, and washed in water and in a series of ethanol solutions of different concentration. Dissected body parts were then stored in 100% ethanol and air dried before being mounted to SEM stubs, and gold-coated.

Terminology for skeletal features follows Richards (1977), Ronquist and Nordlander (1989), and Ronquist (1995). Terminology describing surface sculptures follows Harris (1979). Phylogenetic relationships among the taxa were analyzed with gall morphology characters included and excluded, respectively, using parsimony algorithm as implemented in PAUP* version 4.0b8a (Swafford 2002). Heuristic search method was used with 5000 random addition sequence replicates, followed by tree bisection-reconnection branch swapping (other search options were default in PAUP). Calculations of Bremer support for each node (Bremer 1994) were programmed for batch processing using the same options as the tree searching procedure described above. All characters were unordered and unweighted.

Associations with host plant genera were investigated by optimization onto the resulting two best trees *a posteriori* using MacClade version 4.03 (Maddison and Maddison 2001). The number of host plant colonization events was evaluated assuming an ACCTRAN optimization. We evaluated the degree to which host associations were phylogenetically conserved by calculating their unit consistency indices.

Historical biogeography was reconstructed by using DIVA Version 1.2 (Ronquist 2001) according to the dispersal-vicariance optimization method proposed by Ronquist (1997). Dispersal-vicariance analysis reconstructs the ancestral distribution in a given phylogeny without any prior assumptions about the form of area relationships. Speciation is assumed to subdivide the ranges

of widespread species into vicariant components; the optimal ancestral distributions are those that minimize the number of implied dispersal and extinction events (Ronquist 1997). The DIVA method differs from cladistic biogeography in that it allows nonhierarchical area relationships, and is therefore particularly useful when reconstructing the distribution history of groups occurring in areas that have a reticulate palaeogeographic history, such as the Northern Hemisphere (Nordlander et al. 1996 and references therein).

RESULTS

Character analysis.—We identified 43 morphological characters of adult wasps, and four characters of gall morphology (Appendix). Characters 8, 36, 42, and 45–47 were autapomorphic and thus parsimony-uninformative. They were excluded from analysis, but included in the matrix for reference purposes.

Cladistic analyses.—Parsimony analysis of all characters resulted in two equally most parsimonious trees (Length = 167; CI = 0.36; RI = 0.44; Figs. 3a, 3b). The strict consensus tree is shown in Fig. 4. Excluding the single parsimony-informative gall character resulted in the same two most parsimonious trees with no change in tree topologies, and only slight difference in tree statistics (Length = 165, CI = 0.36, RI = 0.44). Inclusion of gall morphology in analysis resulted in better, though slight, support for three internodes (noticeably two basal internodes) of the best trees (Fig. 4). The two most parsimonious trees differ only in the positions of *D. mayri* and *D. cuscutaeformis.*

As is shown in the strict consensus tree, the phylogenetic relationship among the *Diastrophus* species is mostly resolved. As we predicted, the monotypic genus *Gonaspis* is nested within *Diastrophus.* The relationship among *D. mayri, D. cuscutaeformis,* the clade (*D. rubi,* (*D. fragariae, D. tumefactus*)), and the rest of the genus at the base of the phylogenetic tree is not resolved. The two subspecies of *D. kincaidi,*
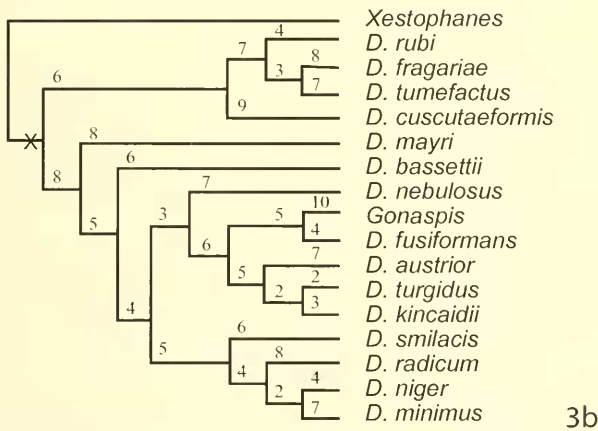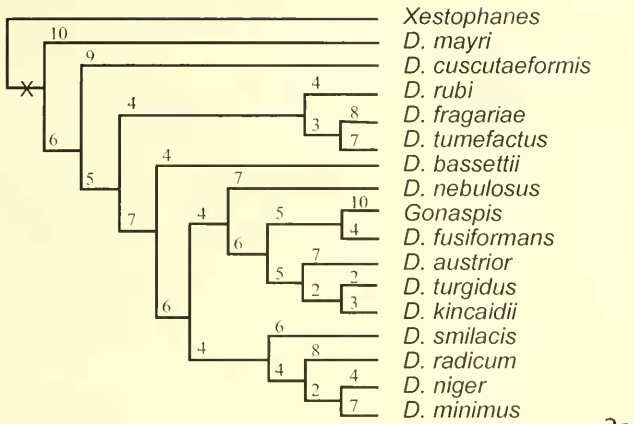
Fig. 3. Two equally most parsimonious trees for *Diastrophus, Gonaspis,* and *Xestophanes,* with the number of apomorphic character changes for each branch indicated (tree length = 167, CI = 0.36, RI = 0.44). Program for phylogenetic analysis was PAUP 4.0 (beta 10) (Swofford 2002). Six characters that are parsimony-uninformative were excluded from the analysis, but were included when calculating the number of apomorphic character changes on terminal internodes.

i.e., *D. k. kincaidi* and *D. k. austrior,* do not appear as sister species in the phylogeny, although they were shown to be closely related.

Optimization of host plant association onto tree #1 (Fig. 5a) requires 6 steps (CI = 0.50) to explain the current pattern. It suggests that the basal species of *Diastro-*

Table 2. Character matrix. Missing data and characters inapplicable to a particular taxon are represented by "-". Polymorphism is by letters "p" (=01), "q" (=02), and "x" (=12). Based on result of phylogenetic analysis, *Gonaspis* is treated in the present study as a synonym of *Diastrophus*, and thus *G. potentillae* = *D. potentillae, sensu lato.*

Taxa	Characters 1	10	21	31	41
Xestophanes	111100001	002117007	1-012-300	010100011	1001001
'Gonaspis'	100100111	11210xxx	p113-1x	xx1311121	0020010
D. austrior	10100C0111	11110001p4	p1p12c000	100110x001	10x0001
D. bassettii	101001120	000101011	0101013	0022102 1	0021001
D. cuscutaeformis	001110-3	0011120070	00-310.	11p110x000	0020011
D. frageriae	014210100	00010_00	00-100 00	1p0110111	7010201
D. fusiformans	130pp1311	11010p101	20-101 11	12p110011	7020001
D. kincaidii	100p010111	11001001	1q111pp00	1p011x1_p	7010001
D. mayri	100020120	100011111	1p01-12q	110110x011	p021001
D. minimus	100110111	11001001	0111011010	00100331	0020001
D. nebulosus	100010110	001012011	1211201011	110010111	0020001
D. niger	00110 x1	1010100107	1p02x11010	100 p03000	00x0001
D. radicum	101111111	10p10q011	1112x11010	112210x000	010p101
D. rubi	01020120	0110111	00-1111200	11px101011	0p10001
D. smilacis	100p010110	00012322110	1112100010	11q200000	p020001
D. tumefactus	100110100	010001117	00-1111002	110010011	p000001
D. turgidus	100p020111	11000p30000	110p21p001	1002102_11	0010001

plus was associated with *Potentilla,* which appears to have been re-colonized twice by more derived species, and that colonization of *Rubus* has occurred once (having been reversed by the recolonizations of *Potentilla* and one shift each to *Fragaria* and *Smilax*). Optimization onto tree #2 (Fig. 5b) requires 7 changes (CI = 0.33) to explain the current pattern, an extra change over the previous scenario and suggests that the association with *Rubus* is less conserved. The associations with the plant genera *Fragaria,* and particularly that with *Smilax,* in both optimizations, are nested in the crown clade of each most parsimonious cladogram. Associations with both *Potentilla* and *Rubus* are slightly more conserved in tree #1 (Fig. 5a), in which the unit consistency indices of each association are 0.25 and 0.20, respectively, as compared with 0.20 and 0.17 in tree #2 (Fig. 5b).

Optimization of plant growth form associations onto tree #1 (Fig. 6a) requires four changes (CI = 0.25) and suggests that the basal species of *Diastrophus* was associated with herbaceous host plants and col-

onization of woody host plants occurred only once, followed by three subsequent reversals to herbaceous hosts. In contrast, optimization onto Tree #2 (Fig. 6b) requires five changes with lower consistency index (CI = 0.20), and is ambiguous about the ancestral state of plant growth form association.

DIVA analysis of the historical biogeography of *Diastrophus* based on the phylogenetic relationship among species depicted in Figs. 3a–b resulted in two equally optimal alternative reconstructions, each suggesting four dispersal events. Based on tree #1, the ancestor of *Diastrophus* was present in both West Palearctic and East Nearctic and a vicariance event split the West Palearctic *D. mayri* from the rest of the genus in East Nearctic (Fig. 7a). A clade later expanded its distribution to West Palearctic, followed by a vicariance event, which gave rise to *D. rubi* in West Palearctic and *D. fragariae* + *D. tumefactus* in East Nearctic. The West Nearctic species *D. austrior* and *D. kincaidii* resulted from independent dispersal events from East Ne-
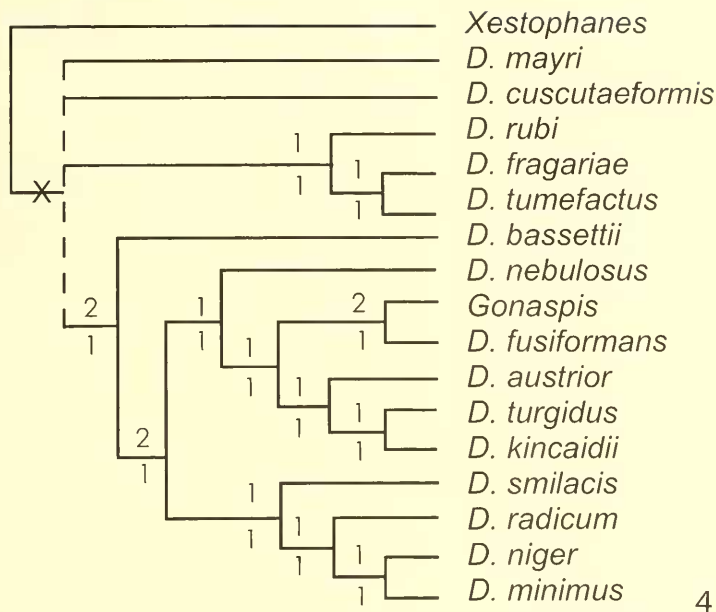
Fig. 4. Strict consensus tree of the two equally most parsimonious trees. Number above and below each branch are Bremer Support values resulting from constrained search with the single informative gall morphology character included and excluded.

arctic followed by subsequent vicariance. The occurrence of *D. fusiformans* is due to recent dispersal. Alternatively (Fig. 7b), the ancestor of *Diastrophus* was present in East Palearctic, and both West Palearctic species, i.e., *D. mayri* and *D. rubi,* are due to dispersal events to West Palearctic followed by a vicariance event. Otherwise the two reconstructions do not differ (Figs. 7a–b).

DISCUSSION

Phylogeny and systematics of *Diastrophus.*—The phylogenies should be viewed as working hypotheses about relationship of the group. Although resolved, the low consistency indices, retention indices, and Bremer values suggest that there is not enough character congruence in our data to render a robustly supported cladogram. External

morphology in this group exhibits considerable homoplasy, possibly as a result of convergence associated with gall-forming life styles. An analysis based on molecular characters may be more informative. Nonetheless, our analysis does provide a provisional phylogeny and a preliminary basis for exploring the macroevolution of host plant associations and historical biogeography.

The phylogenetic position of the monotypic *Gonaspis* as nested within *Diastrophus* is confirmed by all analyses, thus the species *Gonaspis potentillae* (Bassett 1864) is hereby formally transferred to *Diastrophus,* where the species was originally placed. The genus *Gonaspis* was established by Ashmead (1897) based on *Diastrophus scutellaris* Gillette 1891, which
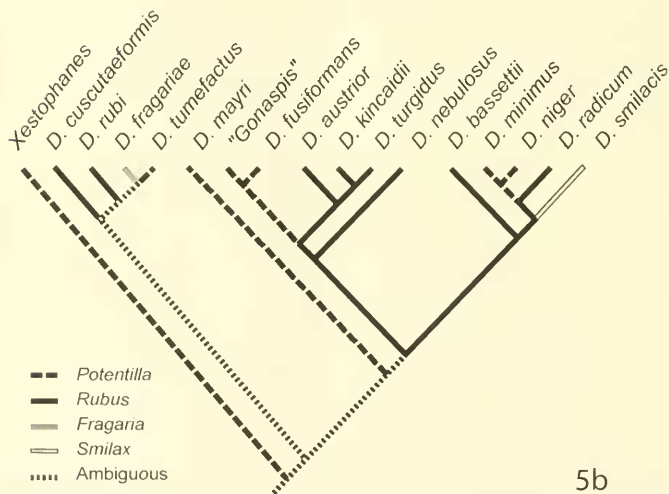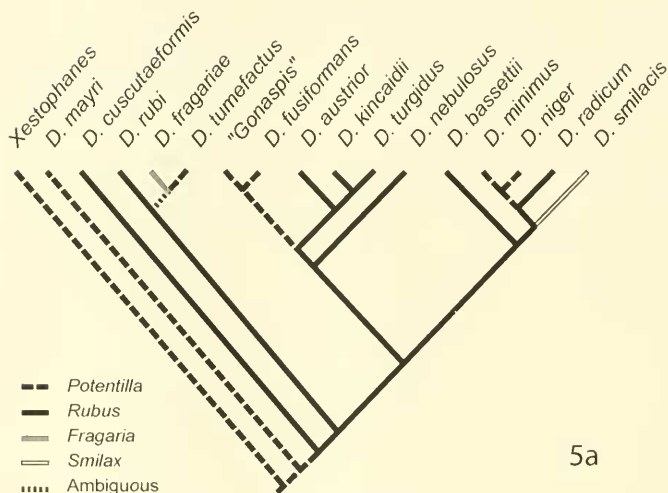
Fig. 5. Macroevolutionary pattern of host plant association in *Diastrophus*, *Gonaspis*, and *Xestophanes* as optimized using MaClade 4.0 onto best tree #1 and best tree #2, respectively.

was considered a junior synonym (Weld 1952), and listed as a variety of, *G. potentillae* by Weld (1959). Weld's first treatment has become accepted (Burks 1979). Therefore, the genus *Gonaspis* Ashmead 1897 is **a new junior synonym** of *Diastrophus* Hartig 1840.

Diastrophus kincaidii austrior, originally described by Kinsey (1922) as a variety of *D. kincaidii* Gillette, 1893, was later treated as a subspecies of the latter by Weld (1959). Burks (1979) listed it as a synonym of *D. kincaidii.* Since the two taxa do not appear as sister species in the cladogram, we thus establish *D. kincaidii* var. *austrior* Kinsey 1922 as a valid species, *Diastrophus austrior* Kinsey 1922. Both *D. kincaidii* and *D. austrior* induce galls on *Rubus parviflorus,* however, *D. kincaidii* is distributed in Washington and Oregon, and *D. austrior* is restricted to California based on known collection records.

Host plant associations and gall features.—Tree #1 suggests that an association with *Potentilla* was the ancestral condition with respect for *Diastrophus,* and was secondarily derived in *D. tumefactus,* and the respective common ancestors of the sister species [*D. minimus* + *D. niger*], and of [*D. potentillae* (= *Gonaspis potentillae*) + *D. fusiformans*]. The position of the species associated with *Smilax* and *Fragaria* suggests relatively recent and unique host shifts. In contrast, tree #2 offers an ambiguous scenario for ancestral host plant associations of *Diastrophus.* This is a result of the shifted position of *D. mayri.* Otherwise the two scenarios (Figs. 5a–b) are similar. In either case, association with *Potentilla* appears to have been labile, with at least three independent origins, whereas association with *Rubus* may be more conserved. In neither scenario did we find a perfectly conserved association with any host plant genus for which there exist more than one associated *Diastrophus* species (i.e., *Potentilla* and *Rubus*).

With regard to the macroevolutionary pattern of association with host plant

growth form, tree #1 gives a more parsimonious and straightforward reconstruction compared with tree #2, which requires one more extra step, has lower consistency, and is uncertain about the ancestral state of host plant growth form association. The first scenario suggests a single event of host shifting from herbaceous to woody hosts. In contrast, the second scenario suggests this only as a possibility, but also suggests another possibility, i.e., colonization to woody hosts has occurred independently at least twice. In either case, reversal from woody hosts to herbaceous hosts has occurred at least twice, contrary to the general pattern in the clade of rosid gall makers, where colonization of woody hosts occurred only once and no reversal were observed (Ronquist and Liljeblad 2001). It is interesting to note the plasticity of the many aspects of *Diatrophus* biology. Most of *Diastrophus* (*sensu lato*) and *Xestophanes* are the only taxa that induce galls on rosaceous host plants among the 156 known species in the tribe Aylacini; all others are associated with advanced herbaceous hosts. *Diastrophus* is the only cynipid genus that has both members that are associated with herbaceous host plants and with woody hosts, contrary to the generally conservative association with plant growth forms in all other cynipid gall makers. As mentioned earlier, all basal cynipid genera are herb gallers, while the host plants of the rosid gallers are all woody. This conservative characteristic of association with plant growth form reaches its extreme in the rosid gallers comprising more than 1,000 species and with no single exception: Diplolepidini (63 species) are associated with *Rosa* (Rosaceae), Eschatocerini (3 species) with *Acacia* and *Prosopis* (Fabaceae), Pediaspidini (2 species) with *Acer* (Aceraceae), and Cynipini (ca 1,000 spp) with *Quercus* (Fagaceae, with a few exceptions associated with other genera of the same plant family; Ronquist 1994, Liljeblad and Ronquist 1998, Ronquist 1999).

According to phylogenetic study based on both morphology and molecular data,
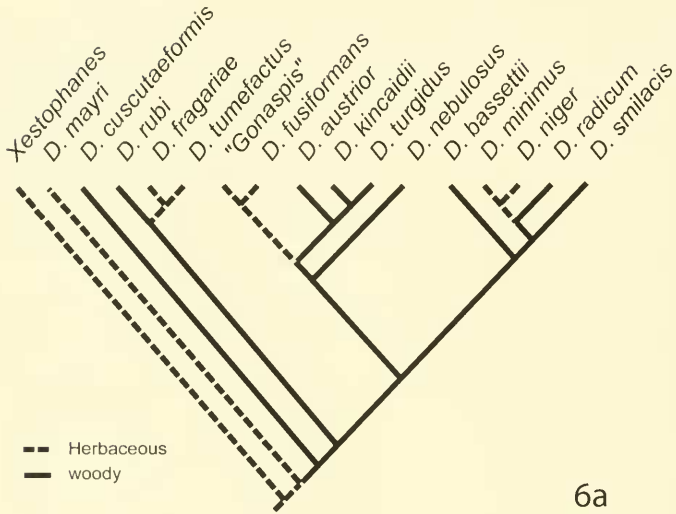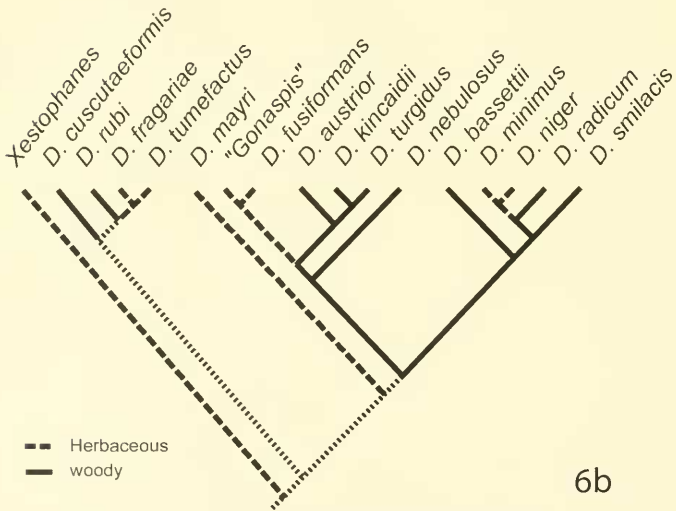
Fig. 6. Macroevolutionary pattern of association of host plant growth forms in *Diastrophus, Gonaspis,* and *Xestophanes* as optimized using MaClade 4.0 onto best tree #1 and best tree #2, respectively.

the plant genera *Fragaria, Potentilla,* and *Rubus,* associated with *Diastrophus* belong to a monophyletic clade with another genus *Rosa* in the plant family Rosaceae (Evans 1999). Therefore, species of *Diastrophus* are still conservative in their host plant as sociation, with the exception of *D. smilacis.* Once the ancestral species of the genus colonized any of the genera *Fragaria, Potentilla,* or *Rubus,* it would have become less restrictive for subsequent colonization of other genera in the same clade, from herbaceous to woody host or vice versa, leading to repeated reversal events from woody hosts to herbaceous hosts (Figs. 6a–b) and thus the relatively high species diversity of the genus compared to most other herb galling cynipid genera (cf., Ronquist and Liljeblad 2001).

Like all other herb gallers, *Diastrophus* species mostly induce simple swellings on stems with multi-chambers. However, several species show derivations: *D. cuscutaeformis* makes clusters of singular, detachable, and seed-like galls. *D. fragariae* makes galls on leaf petioles of strawberry (*Fragaria virginiana* Duch.), and *D. radicum* and *D. bassettii* make underground galls on the roots of *Rubus.* Indeed, the only parsimony-informative gall character we scored is the location of the galls, below or above ground, and that only by virtue of two species bearing one of two alternative states (underground gall-forming). However, this behavior appears convergent in each most parsimonious tree, considering that the galls of *D. bassettii* are stem galls and are not always below ground level (Beutenmueller 1909), and hence was coded as polymorphic in our analysis. Our phylogenetic analysis supported this by showing the *D. bassettii* and *D. radicum* are not sister species (Figs. 5a–b).

Biogeography.—The biogeographical re construction of speciation pattern based on tree #1 (Fig. 7a) suggested that the ancestral species of *Diastrophus* was distributed in both West Palearctic and East Nalearctic, with a basal vicariance event that separated

D. mayri and the rest of the genus. In this scenerio, *D. rubi* originated from a vicariance event that followed dispersal of its common ancestral species with *D. fragariae* and *D. cuscutaeformis,* from East Nearctic to West Palearctic. Alternatively, re construction (Fig. 7b) based on the second best tree suggests that the common ancestor of *Xestophanes* and *Diastrophus* occurred in both West Palearctic and East Nearctic and had later become split through a vicariant event. Two subsequent dispersal events gave rise to the two West Palearctic *Diastrophus* species.

The two biogeographical reconstructions do not differ otherwise, and all other species involved have an East Nearctic distribution, except *D. austrior, D. kincaidii,* and *D. fusiformans. Diastrophus austrior* and *D. kincaidii* are only found in California, Oregon, and Washington, while *D. fusiformans* is also found in the East Nearctic. The host plant of the two species, *Rubus parviflorus* Nutt.), has a distribution in western North America and not beyond the Great Lake areas to the east. *Diastrophus fusiformans* makes galls on *Potentilla,* and its wider distribution may be attributable to the distribution of its host plant. The terminal position of this clade indicates that colonization of West Nearctic has been relatively recent event(s).

In a forthcoming study, Engel et al. (pers. comm.) suggested that *Aulacidea succinea,* a fossil species described from Baltic amber (Eocene, ca 45 MYA) by Kinsey (1937) and transferred therein to a newly erected genus, is closely related to the genera *Xestophanes* and *Diastrophus* (*sensu lato*). Therefore, the presence of *Diastrophus* possibly dates as far back as 45 million years ago in the Eocene. Since the oldest known cynipid fossils with putative associations with *Rosa* is from the Oligocene (33–23 MYA; Cockerell 1921, Ronquist 1999) and the oldest fossils that are definitely cynipine galls are on *Quercus* from Middle Miocene (ca. 15–12.5 MYA; Waggoner and Poteet 1996, Waggoner 1999), the species of *Dias-*
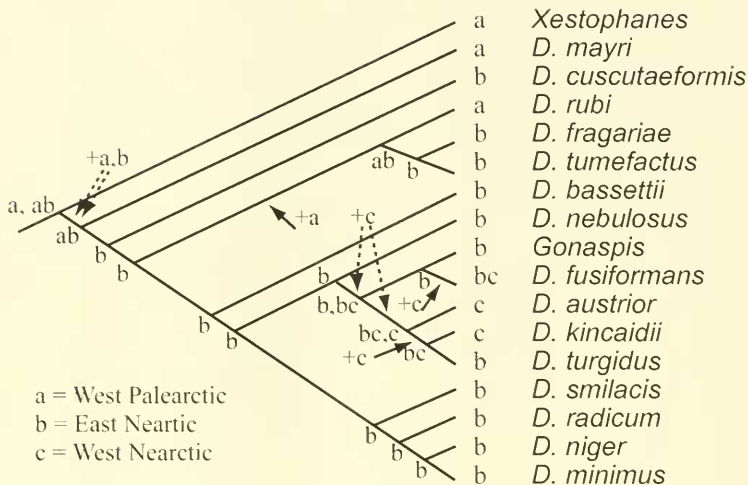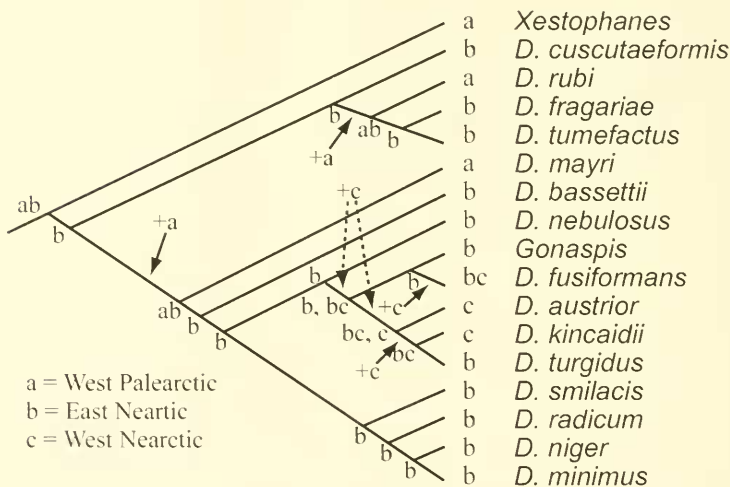
Fig. 7. DIVA reconstruction of historical biogeography for *Diastrophus* based on tree one and tree two, respectively. Search options were default in DIVA. Solid arrows are unambiguous dispersal events and dashed arrows indicate competing alternatives of dispersal events.

trophus associated with woody *Rubus* are likely to represent the first pioneers in Cynipidae in the woody world, although leading to a different clade from the by far more speciose clade comprising Diplolepidini, Eschatocerini, Pediaspidini, and Cynipini (Liljeblad and Ronquist 1998).

Definite evidence of the early appearance of Rosaceae in fossil records is not known before Middle Eocene (ca 42–50 MYA) (Stewart 1983), therefore the existence of the genus *Diastrophus* much earlier is unlikely. Land connections between North America and Europe probably existed until the Oligocene (37 Ma) (Hallam 1981). The presence in Beringia of temperate plants such as members of *Potentilla* was possible until the latest Tertiary or even Quaternary when glaciations cut off dispersal of temperate plants through the Bering land bridge (Tiffney 1985). Thus, it is difficult to speculate by which route, North America—Europe land connection or Beringia, earlier species of *Diastrophus* expanded their distribution. The collection of *Synophromorpha* from Japan as discussed earlier and from southern China (Liu, unpubl. data) indicates that *Diastrophus* is very likely to be present there as well. Inclusion of those species in future analysis certainly will present a clearer picture of the historical biogeography of the genus.

ACKNOWLEDGMENTS

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APPENDIX

List of Characters

I. Structural characters

 1. *Head width compared to mesosoma
    width:* (0) not or only slightly wid-
    er, (1) distinctly wider.
 2. *Apparent number of female anten-
    nal segments:* (0) 13, (1) 14.
 3. *Length of third antennal segment in
    relation to the fourth:* (0) distinctly
    longer, (1) subequal.
 4. *Shape of face in frontal view:* (0)
    high, with height about four-fifths
    width of face, (1) transverse, with

height three quarters or less of width of face.

5. Shape of gena in frontal view below eye: (0) straight in upper three fourths and distinctly curved ventrally, (1) smoothly curved.

6. *Ventral projection of clypeus:* (0) not projected, (1) distinctly project ed ventrally, forming broad emargination with ventral margin of malar space, (2) strongly projected ventrally, tonguelike.

7. *Height of pronotal plate:* (0) height about one-half width, (1) height at least two-thirds width.

8. Notauli: (0) incomplete; (1) complete.

9. *Width of notauli:* (0) narrow throughout, (1) conspicuously widened posteriorly, (2) wide throughout.

10. *Distance between notauli at the posterior margin of mesoscutum:* (0) ≥3 times width of notaulus at posterior margin, (1) not or slightly more than 2 times width of notaulus at posterior margin.

11. Distance between anterior ends of notauli or signal extensions of notauli relative to that between posterior ends of notauli: (0) about two times as wide anteriorly as posteriorly, (1) three to four times as wide anteriorly as posteriorly.

12. *Anterior extension of median me soscutal impression:* (0) absent, (1) present.

13. *Parascutal carinae in dorsal view:* (0) smoothly curved, not raised lateroposteriorly, (1) expanded lateroposteriorly into a rounded angle, not raised posteriorly, (2) expanded lateroposteriorly into an acute, distinctly raised posterior angle.

14. *Parapsidal signa:* (0) absent, (1) present.

15. *Anteroadmedian signi on mesoscutum:* (0) present, (1) absent.

16. *Posterior projection of scutellum:* (0) absent, scutellum gradually sloped and rounded posteriorly, (1) scutellum distinctly projected posteriorly into an apical, broadly truncate process, (2) scutellum conspicuously projected posteriorly into an apical, narrowly truncate process (Fig. 2f).

17. *Slope of posterior projection of scu tellum in lateral view:* (0) distinctly curving downward toward posterior apex, (1) almost flat.

18. *Extension of longitudinal linear ca rinae on dorsal surface of scutellum:* (0) absent completely or absent in posterior third of scutellum, (1) extending to end of dorsal surface.

19. *Anterior connection of longitudinal linear carinae on dorsal surface of scutellar disk:* (0) absent, (1) forming concentric semicircles at the anterior end of scutellar disk.

20. *Median depression of scutellum ex tending posteriorly from septum:* (0) absent, (1) only over anterior portion of scutellar disk, (2) throughout scutellar disk.

21. *Width of lateral bars:* (0) narrow (less than half length), (1) wide (greater than half length).

22. *Presence of areolet on forewing:* (0) absent, (1) present, but not defined by tubular vein(s), (2) present, defined by three distinct veins.

23. *Shape of areolet in forewing:* (0) present, less than 4 times width of anal vein, (1) present, equal to or wider than 4 times width of anal vein.

24. *Length of bulla in forewing:* (0) absent, (1) present, nearly as wide as vein width and less than two times vein width, (2) present, at least twice as wide as vein width.

25. *Size of abdominal tergum 3 in re lation to post-petiolar metasoma in lateral view:* (0) more than half of metasoma, (1) about half the meta-

soma, (2) less than one-third meta-somal area.

II. Surface sculpture

26. *Coarseness of radiating strigulation in malar space:* (0) coarsely substrigulate, (1) finely strigulate.

27. *Extension of strigulation from malar space upward to lower face:* (0) this pattern of strigulation not continued upward to lower face, (1) strigulation continued upward to bases of antennae, (2) pattern of strigulation is bent as it extends upward.

28. *Sculpture on elevated median quadrangular area between antennae and clypeus:* (0) mostly glabrous, (1) coriaceous, (2) mostly strigulate.

29. Sculpture on surface of clypeus: (0) glabrous, (1) strigulate near epistomal sulcus, (2) embossed, (3) with a spherical elevation.

30. *Sculpture of vertex and upper face:* (0) glabrous, (1) coriaceous to slightly strigulate.

31. *Sculpture of gena, especially posteriorly:* (0) glabrous, (1) strigulate, (2) coriaceous.

32. *Upper occiput:* (0) glabrous, (1) finely, transversely strigulate, (2) coriaceous.

33. *Surface sculpture of pronotal plate:* (0) mostly glabrous with sparse puctures, (1) strigulate with some punctures, (2) mostly punctate.

34. *Pubescence on pronotal plate:* (0) absent to sparse, (1) present in two dorso-lateral patches, (2) short setae evenly scattered.

35. *Degree and strength of diagonal carinae on lateral surface of pronotum:* (0) mostly glabrous, carinate only toward margins, (1) largely diagonally carinate.

36. *Surface sculpture of mesoscutum:* (0) glabrous, (1) coriaceous.

37. *Surface sculpture of mesopleuron:* (0) entirely glabrous, (1) mostly strigulate, (2) glabrous with a few central striae.

38. *Surface sculpture at bases of scutellar fovea:* (0) generally glabrous, (1) entirely strigulate-reticulate or rugulose, (2) some strigulation posteriorly.

39. *Sculpture on dorsal surface of axilla:* (0) glabrous except posteriorly, (1) rugulose, (2) coriaceous.

III. Body color and pubescence

40. *Color of head and mesosoma:* (0) brown to reddish brown entirely or in part, (1) deep dark brown to black.

41. *Color of metasoma:* (0) brown to reddish brown entirely or in part, (1) deep dark brown to black.

42. *Infuscation of membranous areas of forewings often appearing as a linear streak in Rs cell:* (0) absent or very faint, (1) present.

43. *Presence of infuscation of forewings near veins (especially R1 and 2r):* (0) absent, (1) present, but indistinct, infuscate area usually less than vein width, (2) infuscation dark and distinct, usually as wide as vein.

IV. Gall Features

44. *Position of gall growth:* (0) Above ground, (1) below ground.

45. *Plant tissues affected by gall growth:* (0) stem, (1) root, (2) leaf.

46. *Gall structure effects on plant surface:* (0) internal and integral, (1) external and detachable.

47. *Surface of gall indicating internal chambers:* (0) individual, (1) as single entire group, (2) multiple galls in close proximity.